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Putative morphometric evidence of the presence of *Acipenser naccarii* Bonaparte, 1836 in Iberian rivers, or why ontogenetic allometry needs adequate treatment

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ABSTRACT

I have extended my initial analyses (Rincón, 2000) of a published morphometric data set of sturgeons caught in Iberian rivers and from a fish farm, claimed to prove the native status of both *Acipenser naccarii* Bonaparte, 1836 and *Acipenser sturio* L., 1758 in the Guadalquivir basin (Garrido-Ramos *et al.*, 1997), by applying the same uni- and multivariate techniques for allometric adjustment to a database expanded with further *A. naccarii* specimens. As previously, neither *log-log* scatterplots of head measurement vs. total length, nor graphical representation of the scores of individual specimens on the principal components (PC) extracted by PCAs performed on the covariance and correlation matrix, respectively, offered any suggestion of the existence of two morphologically dissimilar groups within wild Iberian sturgeons. In addition, the relatively weak (in terms of total variance accounted for) gradient in snout width that separated the one farmed Adriatic sturgeon from the wild fish found in my previous work (Rincón, 2000) was again detected, now clear and distinct, by all those techniques. Furthermore, a DFA showed that such dissimilarity produced almost perfect, highly statistically significant discrimination between farmed and wild fish, while there was no significant gradient separating the two supposed groups of wild Iberian sturgeons. Examination of the two composite variables that Garrido-Ramos *et al.* (1997) used to separate those groups in the extended database confirmed that they were negatively affected by ontogenetic allometry, therefore leading to the ascription of large and small specimens to different groups. I conclude that there is no morphological evidence to support the claimed autocthonous status of *A. naccarii* in the Iberian Peninsula.

Key words: Morphometrics, size correction, PCA, sturgeons.

RESUMEN

Supuesta evidencia morfométrica de la presencia de* Acipenser naccarii *Bonaparte, 1836 en ríos ibéricos, o por qué la alometría ontogénica necesita un tratamiento adecuado

He ampliado mi análisis inicial (Rincón, 2000) de los datos publicados sobre la morfometría de esturiones procedentes de aguas de la península Ibérica y de una piscifactoría, presentados como prueba del carácter nativo tanto de Acipenser naccarii *Bonaparte, 1836 como de* Acipenser sturio *L., 1758 en la cuenca del Guadalquivir (Garrido-Ramos* et al.*, 1997), aplicando las mismas técnicas uni- y multivariantes de ajuste de alometrías a una base de datos ampliada con un mayor número de ejemplares de* A. naccarii. *Como ya ocurrió, la representación gráfica de las diversas medidas cefálicas frente a la longitud total (todas transformadas a logaritmos) y de los componentes principales extraídos por ACP, realizados usando tanto la matriz de covarianza como la de correlación, no ofrecieron indicación alguna de la existencia de dos grupos morfológicamente distintos dentro de los esturiones capturados en aguas de la Península. Además, el relativamente débil (en cuanto a proporción de la varianza total que asumía) gradiente en la anchura del*

morro que separaba al único ejemplar de piscifactoría de los esturiones salvajes que hallé (Rincón, 2000), fue identificado de nuevo, ahora evidente y nítido, por todas las técnicas anteriores. Finalmente, un AFD mostró que tal diferencia permitía una casi perfecta, altamente significativa, distinción entre los especímenes de piscifactoría y los salvajes, mientras que no hubo gradiente morfométrico estadísticamente significativo que separase los supuestos dos grupos de esturiones salvajes ibéricos. El examen en la base de datos ampliada de las dos variables compuestas que Garrido-Ramos et al. *(1997) usaron para distinguir tales dos grupos, confirmó que ambas se hallan gravemente influidas por la alometría ontogenética, llevando, por tanto, a la asignación de los ejemplares grandes o pequeños a grupos distintos. Concluyo, pues, que no hay evidencia morfológica alguna que apoye el supuesto carácter de especie autóctona de* A. naccarii *en la península Ibérica.*

Palabras clave: *Morphometría, corrección del tamaño, ACP, esturiones.*

INTRODUCTION

The Adriatic sturgeon, *Acipenser naccarii* Bonaparte, 1836, has been generally considered restricted to the Adriatic Sea, particularly its northern area, and the river basins draining into it (Holčík, 1989). Recently, however, Garrido-Ramos *et al.* (1997) have stated that individuals of the species comprise a substantial part of the sturgeons collected in the Iberian Peninsula (and in the Guadalquivir River in particular) from the end of the last century until the early 1980s; this is in direct conflict with the widely-held view that the Atlantic sturgeon *Acipenser sturio* L., 1758 is the only native sturgeon species in the Iberian Peninsula (Classen, 1944; Almaça, 1988; Doadrio, Elvira and Bernat, 1991; Elvira, Almodóvar and Lobón-Cerviá, 1991; Elvira and Almodóvar, 1993; Pereira, 1995). From this, Garrido-Ramos *et al.* (1997) moved on to claim the status of autochthonous, endangered species for *A. naccarii* in the Guadalquivir and, consequently, to demand the implementation of recovery plans for the species which entail its stocking in that area (Garrido-Ramos *et al.*, 1997; Ruiz-Rejón, Hernando and Domezain, 1998).

However, these assertions have been negated by a number of authors who have strongly criticised the evidence that allegedly substantiates them (Doukakis *et al.*, 2000; Elvira and Almodóvar, 2000; Rincón, 2000; Almodóvar, Machordom and Suárez, 2000). Such putative evidence is both morphometric and genetic. The genetic findings of Garrido-Ramos *et al.* (1997) could not be replicated by Doukakis *et al.* (2000) and have been directly contradicted by Almodóvar, Machordom and Suárez (2000). I have shown elsewhere that their morphological results are an artifact caused by their application of an inadequate methodology (Rincón, 2000) unable to separate ontogenetic and evolutionary allometry (Cock, 1966; Klingenberg, 1996).

The failure of Garrido-Ramos *et al.* (1997) to distinguish between allometry caused by growth processes within a taxon from that originated by phylogenetic variation between taxa, and to efficiently account for their effects, led them to misidentify large specimens of *A. sturio* as *A. naccarii*. The application of routine univariate and multivariate morphometric techniques, which adequately deal with allometry to the same data set, found that, indeed, there was only one group of morphometrically similar sturgeons among the specimens caught in Iberian rivers and traditionally identified as *A. sturio*. Furthermore, they detected the morphological dissimilarity of the only specimen *a priori* known to be *A. naccarii* (a sturgeon from a fish farm) present in the sample (Rincón, 2000).

However, this very scarcity of true *A. naccarii* in the Garrido-Ramos *et al.* (1997) database determined that gradients reflecting interspecific differences accounted for relatively little of the total variance of the sample in multivariate morphometric space. Thus, size-related gradients were magnified by default. Therefore, further assessment of the relative performance of the techniques used by Garrido-Ramos *et al.* (1997) and my previous work (Rincón, 2000), respectively, under different conditions seems warranted. To do this, I have added data from eight new *A. naccarii* to the original 25-specimen database that both articles examined, and then I have applied to this enlarged database the same common multivariate and univariate techniques for allometric correction I had previously used (Humphries *et al.*, 1981; Bookstein *et al.*, 1985; Reist, 1985, 1986; Klingenberg, 1996).

Specifically, the current paper seeks to answer the following questions: (1) how are my conclusions (Rincón, 2000) affected by the application of the same methodology to a sample where *A. naccarii* abundance is increased?; and (2) do the criteria in Garrido-Ramos *et al.* (1997) correctly distinguish between the two species over their entire size range, as claimed by those authors?

MATERIALS AND METHODS

Data

Table 1 in Garrido-Ramos *et al.* (1997) is one source of the morphometric measurements used in my analyses. It reports the values of the following six variables: (1) total length (Tl, cm); (2) distance from the tip of the snout to the base of the barbels (A, cm); (3) distance from the base of the barbels to the cartilaginous arch of the mouth (C, cm); (4) distance from the tip of the snout to the cartilaginous arch of the mouth (F, cm); (5) width of the snout at the point of barbel insertion (B, cm); and (6) distance from the tip of the snout to the frenulum (LFR, cm). From those measurements they also derive the subtraction C - A and the ratio F:B (Garrido-Ramos *et al.*, 1997, table 1, figure 2A,B). The values of these eight variables are given for 25 individuals (LFR is missing in specimen EBD-8174, number 2 in table 1 of Garrido-Ramos *et al.*, 1997) of which, one (PSN-1) comes from a farm-raised *A. naccarii* from an originally Italian stock. Therefore, the specific identity of PSN-1 seems certain. All the other are wild fish and could, hypothetically, comprise both *A. naccarii* and *A. sturio*.

To ensure comparability, morphometric data have been entered in subsequent calculations as they appear in table 1 of Garrido-Ramos *et al.* (1997) and have not been altered, save for the correction of obvious transcription or typographical error: e.g., the published measurements for specimen EBD-8174 are A = 5.20; B = 11.40; C = 12.60; F = 12.60; C - A = 2.20; and F:B = 1.14. I have inferred that the correct value for C should be 7.40, which I have used in further analyses. Garrido-Ramos *et al.* (1997) provide no information on their measuring protocols, but from their figure 2A,B it is clear that F should be equal to A + C. However, in some specimens (MUC1, PSN1), F is slightly smaller than A + C (e.g. MUC1; F = 10.60 cm; A + C = 11.00 cm) while in others F is larger (SE-1, SE-2, SE-3, F = 4.60, 4.30, 3.20 cm; A + C = 3.90, 3.45, 2.56 cm, respectively). Again, to maintain comparability, and given that the reported values of F are not always a linear combination of C and A, I have retained F in further analyses.

The cases of the subtraction C - A and the ratio F:B, the main basis of the claims made in Garrido-Ramos *et al.* (1997), deserve more detailed comment. Elsewhere (Rincón, 2000), I have shown that (1) they are both significantly influenced by overall size (represented by Tl in our case) and, therefore, cannot be used as substitutes of the standard statistical techniques employed to deal with the effects of size and allometric growth; and (2) as C - A and F:B do not represent distances between two identifiable morphological features, uni- or multivariate procedures for allometric correction involving them are no longer rooted in the model of animal ontogenetic development and growth first proposed by Huxley (1932), and which has received abundant further empirical and theoretical support since then.

For all of the above, I consider that the use of C - A and F:B, even with some form of size-correction, must be avoided. Moreover, preliminary multivariate analyses carried out including or excluding C - A (correcting for size effects) produced similar results and identical conclusions. Consequently, I here present analyses performed using the original measurements exclusively, save for further exploration of the properties and behaviour of C - A and F:B across a wider *A. naccarii* size range.

The remaining morphometric data were obtained from eight *A. naccarii*, 21.7 to 131.7 cm in Tl (table I), and coming from the same fish farm as

Table I. Morphometric data from the specimens of *A. naccarii* from the Sierra Nevada fish farm housed in the scientific collections of the Príncipe Alberto I de Mónaco Aquatic Ecology Station (PSN-2 to 5) and of the Doñana Biological Station (PSN-6 to 9), Seville

	Tl	A	B	C	F	LFR	C - A	F:B
PSN-2	21.70	1.32	1.86	1.18	2.50	4.73	-0.14	1.34
PSN-3	23.00	1.54	2.03	1.05	2.59	5.08	-0.49	1.28
PSN-4	75.50	2.60	5.90	3.40	6.00	13.20	0.80	1.02
PSN-5	78.00	2.75	6.30	3.45	6.20	14.00	0.70	0.98
PSN-6	124.00	3.50	8.00	3.95	7.45	21.50	0.45	0.93
PSN-7	131.70	3.70	8.90	4.70	8.40	20.50	1.00	0.94
PSN-8	123.00	3.20	7.50	3.30	6.50	16.00	0.10	0.87
PSN-9	49.50	2.15	3.31	2.18	4.33	8.80	0.03	1.31

PSN-1. I will henceforth refer to them as PSN-2 to PSN-9. Those specimens are housed in the scientific collections of the Príncipe Alberto I de Mónaco Acuatic Ecology Station (PSN-2 to 5) and of the Doñana Biological Station (PSN-6 to 9), both in Seville, but they have not been assigned catalogue numbers yet. Their six original morphometric variables (Tl, A, B, C, F, LFR) were measured and C - A and F:B were also calculated. See Elvira and Almodóvar (2000) for details on measurement procedures.

Data analysis

As in Rincón (2000), I have approached the data as a "problem sample" in which one or more groups of morphologically similar individuals may be present. Therefore, I have relied on exploratory techniques not requiring *a priori* group ascription to identify the patterns of morphometric variation within the sample. Then, these patterns can be contrasted against explanatory hypotheses, such as allometric growth or presence of several taxa in the sample, which, obviously, need not be mutually exclusive. Morphometric dissimilarity of the magnitude claimed by Garrido-Ramos *et al.* (1997) should be reflected in the results of one or more of the different techniques (ideally, all of them), if it is not merely a procedural artifact. See Smith (1973), Neff and Smith (1979), Humphries *et al.* (1981), or Humphries (1984) for applications of such an approach.

As recommended by Marcus (1990), as an initial step in my analyses I produced *log-log* bivariate scatterplots of A, B, C, F and LFR against Tl (not shown) and inspected those representations in search of any spatial arrangement of specimens that could be interpreted as evidence suggestive of the presence of two or more groups of sturgeons in the sample (e.g. spatially disjunct clouds of points in the bivariate plane). The plots did indeed show a spatial disposition indicating that the sample may contain more than one group of morphologically similar fish, although they all exhibited a common pattern of ontogenetic allometric covariation: all *log*-transformed head measurements appeared to increase linearly with *log* length, longitudinal measurements doing so more slowly than transversal ones, in all groups, but the details of the relationship seemed group-specific.

In such a situation, the common model (Huxley, 1932) of allometric growth is applicable. According to it, the formula for simple allometry between two morphological trait measurements *x* and *y* (see also Klingenberg, 1996) is

$$y = b\, x^{\alpha} \qquad [1]$$

very often linearised through *log*-transformation as:

$$log\, y = log\, b + \alpha\, log\, x \qquad [2]$$

where α and b are constants. The constant b is frequently called an allometric coefficient.

I next applied a commonly used method for univariate size correction to the values of A, B, C, F and LFR. I employed the equation

$$log\, y_{adj} = log\, y - \alpha\, (log\, Tl - log\, Tl_M) \qquad [3]$$

where y_{adj} is the adjusted value of variable y, α is as in equations [1] and [2] and was estimated as the pooled slope of the *log-log* regression of variable y against Tl, and Tl_M is the grand sample's mean total length. This technique has been employed often (Ihssen *et al.*, 1981; Baumgartner, 1995; Hawkins and Quinn, 1996) and it has been discussed and preferred by Reist (1985, 1986) to other univariate methods of allometric adjustment. The use of a univariate adjustment is further justified because Tl is strongly correlated with generalised multivariate size in the present data set (correlation between *log* Tl and PC1 extracted by a Principal Components Analysis using the covariance matrix = 0.97; N = 32; $p < 0.00001$; see below for details), and the number of variables involved is small (Humphries *et al.,* 1981). Total length was excluded from subsequent analyses carried out on the adjusted variables (henceforth, $log\, A_{adj}$, $log\, B_{adj}$, $log\, C_{adj}$, $log\, F_{adj}$, $log\, LFR_{adj}$).

I used PCA to explore the distribution of specimens in multivariate morphometric space. PCA is a valuable technique in situations such as the present one because it is not biased by information about group membership (Humphries *et al.,* 1981; Humphries, 1984). I used the covariance matrix of the *log*-transformed values of Tl, A, B, C, F, and LFR to extract the Principal Components (PCs) that ideally represent the major axes of multivariate morphometric variation (Humphries *et al.,* 1981; Bookstein *et al.,* 1985; Rohlf and Bookstein, 1987). Through use of *log* transformation and the covariance matrix allometries are preserved, the geometric space is not distorted, and the original variables

influence the analysis according to their variance (Jolicoeur, 1963; Bookstein *et al.,* 1985).

These properties are desirable for two reasons. The first is that size is often the variable with the highest variance in morphometric data sets and, therefore, the first PC extracted (PC1) is often a general size factor. Subsequent PCs account more for shape differences, but will include size information not accounted for by PC1. The inspection of PC structure through examination of loadings and scoring coefficients of the original variables on them will indicate the extent of this. Secondly, when the length of the eigenvector is scaled to unity (as done here), the scoring coefficients convey direct information on the bivariate allometric relationships between the original variables (Humphries *et al.,* 1981; Bookstein *et al.,* 1985; Rohlf and Bookstein, 1987; Klingenberg, 1996).

Such a utilization of PCA is common in the ichthyological literature (Smith, 1973; Neff and Smith, 1979; Wood and Bain, 1995), including sturgeon systematics (Mayden and Kuhajda, 1996). Those references show how, when several groups are present in a sample, they are arranged as clouds of points spatially separated along the morphometric gradients that distinguish them. Such a disposition is what we would expect to see replicated if the current sturgeon sample contained more than one species. On the other hand, we would expect continuous distribution in multivariate morphospace if the sample comprises only one taxa.

When, as in our case, the ordination of specimens in morphospace is as interesting as the patterns of allometry, the use of the correlation matrix for PC extraction is justified (Klingenberg, 1996). However, unlike the situation with the covariance matrix, it would no longer be possible to derive information about allometries from the PC coefficients (Pimentel, 1979; Klingenberg, 1996). Consequently, I have carried out a PCA using the correlation matrix of the five size-adjusted variables to provide additional perspective and complementary insight and contrast to the results of the previous PCA from the covariance matrix.

Finally, I have assessed multivariate morphometric dissimilarity among groups of fish through Multivariate Analysis of Variance (MANOVA) of the five size-adjusted variables (Rice, 1990; Scheiner, 1993; see Wood and Bain, 1995 for an ichthyological example). Those variables that exhibited significant differences and, therefore, potential for among-group discrimination, were then subjected to a Discriminant Factor Analysis (DFA). Both techniques require *a priori* ascription of specimens to groups. Thus, I have distinguished between sturgeons from the Sierra Nevada fish farm, wild fish identified as *A. naccarii* by Garrido-Ramos *et al.* (1997), and wild fish identified as *A. sturio* by those same authors. The farm vs. wild origin distinction is not a morphometric criterium and, consequently, introduces no circularity into the procedure. However, Garrido-Ramos *et al.* (1997) separated *A. sturio* and *A. naccarii* according primarily to the value of C - A and, secondarily, F:B. While neither parameter has been used in my analyses, I have employed the size-adjusted measurements from which they derive. Hence, the information whose between-groups differences are explored may not be totally independent from that used for group assignment in the case of wild fish. However, I believe the analysis is still valid and useful because (1) I have used the size-adjusted variables and C - A and F:B where obtained from the original measurements and uncorrected themselves; and (2) the hypothetical bias the procedure may introduce would be towards magnification of differences among wild fish and minimization of those between farmed fish and the supposed wild *A. naccarii*. Therefore, results running against these trends are reliable, at least in this regard.

RESULTS AND DISCUSSION

Head allometry

Log-log scatterplots of A, B, C, F and LFR against Tl showed some separation between the *A. naccarii* from the fish farm and the *A. sturio* and putative *A. naccarii* caught in Iberian rivers, but provided no evidence of the existence of several groups within the latter (figure 1). They showed larger snout-barbel (A), barbel-mouth (C) and snout-mouth (F) distances and smaller snout width (B) than farmed fish of comparable size. LFR was similar for both groups (figure 1).

The spatial distribution of wild-caught fish in the bivariate planes appeared to be continuous, and adequately explained by allometric growth and normal interindividual variation (figure 1). Specimen AVG, identified as *A. sturio* (Garrido-Ramos *et al.*, 1997), seemed, however, an apparent

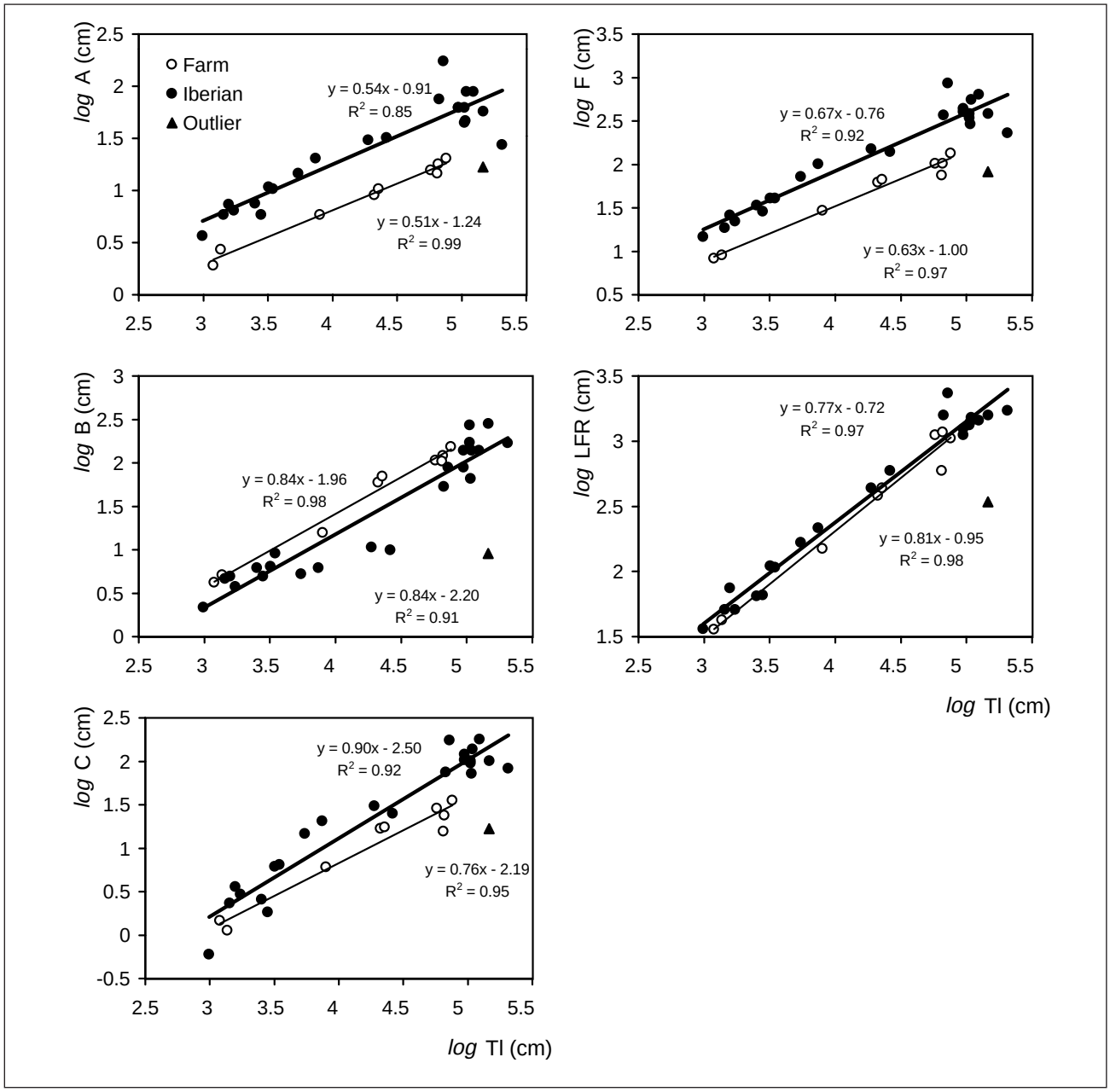

Figure 1. Bivariate *log-log* scatterplots and regressions of sturgeon cephalic morphometric traits (A, B, C, F, LFR, cm) against total length (Tl, cm). Open circles: *A. naccarii* from the fish farm; solid circles: wild-caught Iberian sturgeons; solid triangle: outlier (excluded from calculations)

exception. Its values for all cephalic morphometric traits were much smaller than would be expected in a fish of its size and it appeared as a clear outlier (figure 1). Therefore, it was excluded from the characterization of head allometry in the sample and those further procedures using the size-adjusted variables (i.e. fitting equation 2 to the data, PCA from the correlation matrix), but it was retained in exploratory analyses such as the PCA utilising the covariance matrix.

The allometric model (equation 2) showed a very good fit to the data (table II). Fish size, as measured by Tl, explained a high proportion of the variation in morphometric traits (85-98 %), the allometric coefficients (a) were all highly significant (i.e. different from zero), their estimates accurate (as shown by low standard errors), and the residuals sums of squares were small, as indicated by the high F values of the regressions (table II). Inspection of residual plots showed no apparent

Table II. Allometric coefficients (α) and parameters of the corresponding regression of *log*-transformed cephalic morphometric measurements against *log* Tl for both farmed *A. naccarii* and wild Iberian sturgeons

Variables	Group	R^2	F (df)	α	t (df)	p
log A	wild	0.85	117.65 (1,21)	0.5404	10.85 (21)	< 0.0001
	farm	0.98	487.35 (1,7)	0.5124	22.08 (7)	< 0.0001
log B	wild	0.91	202.93 (1,21)	0.8438	14.25 (21)	< 0.0001
	farm	0.98	390.35 (1,7)	0.8434	14.25 (7)	< 0.0001
log C	wild	0.92	257.73 (1,21)	0.9030	16.05 (21)	< 0.0001
	farm	0.94	121.53 (1,7)	0.7551	11.02 (7)	< 0.0001
log F	wild	0.92	247.73 (1,21)	0.6700	15.74 (21)	< 0.0001
	farm	0.97	271.50 (1,7)	0.6288	16.48 (7)	< 0.0001
log LFR	wild	0.97	663.88 (1,20)	0.7749	25.77 (20)	< 0.0001
	farm	0.98	286.93 (1,7)	0.8147	16.94 (7)	< 0.0001

systematic bias. These results further depict wild-caught Iberian sturgeons as a single morphologically homogeneous group.

All cephalic measurements were negatively allometric relative to total length for both *A. naccarii* from the fish farm and wild sturgeons (all as < 1, table II). This was particularly marked for A and, to a lower degree, F, while B exhibited faster rates of increase with size than either (table II). The allometric trajectories of both groups of fish appeared parallel (figure 1) and an Analysis of Covariance (ANCOVA) with Tl as covariate could find no significant differences between their respective allometric coefficients for either A, B, F, LFR ($F_{1,28}$ = 0.01-0.44; p = 0.51-0.94), or, although closer to significance in this case, C ($F_{1,28}$ = 2.84; p = 0.09). Nonetheless, once the effect of Tl was removed, the two groups were significantly different for A, B, C, and F (ANCOVA, $F_{1,28}$ = 8.85-52.49; p < 0.006 in all cases) due to the different elevations (parameter b in equation 2) of their ontogenetic trajectories (figure 1). LFR showed no significant difference (ANCOVA, $F_{1,28}$ = 1.82; p = 0.19). A, C and F, all longitudinal measurements, were significantly greater in wild specimens, whereas B, a transversal measurement, was smaller (figure 1). This means that farmed *A. naccarii* showed significantly shorter and wider snouts than wild sturgeons of the same size, this being one of the features that distinguishes *A. naccarii* from *A. sturio* (Holčík, 1989), and further indicating the absence of *A. naccarii* in the sample of sturgeons from Iberian rivers.

The net effects of the allometric trends common to both groups were a general reduction of head size relative to overall size with age, and, more significantly in our context, a reduction of pre-oral head dimensions in the longitudinal axis (F), particularly of their pre-barbel component (A), relative to transversal measurements (B). As a result, the snouts of sturgeons in both groups become proportionally shorter and wider relative to younger conspecifics. According to my results, these processes appear parallel in *A. naccarii* and A. sturio (the slopes of the respective regression lines vs. total length are not significantly different). I found no convergence or divergence, and thus the difference in snout width/length between the two species seems to be of similar magnitude throughout their ontogenetic development, at least while there is overlap in size, as *A. sturio* seems to grow to larger sizes than *A. naccarii*.

These findings, however, should be viewed with a measure of caution, given the relatively low number of specimens examined (particularly of *A. naccarii*), and the differences in their preservation methods and history. Nonetheless, they agree well with previous information on allometric variation for *A. sturio* and other sturgeon species. Magnin and Beaulieau (1963) also found a relative shortening of the snout, specifically of the pre-barbel area (i.e. distance A), relative to both total length and head length for both *A. sturio* and the American *Acipenser oxyrinchus* Mitchill, 1815. Similar results have been reported for *Acipenser baerii* Brandt, 1869; *Acipenser gueldenstaedtii* Brandt & Ratzeberg, 1833; *Acipenser ruthenus* L., 1758; *Acipenser nudiventris* Lovetzky, 1828; *Acipenser stellatus* Pallas, 1771; and *Huso huso* (L., 1758) (see chapters on each species in Holčík (1989) and references therein). Therefore, this relative shortening and widening of the head as age and size increase seems to be a general allometric trend in sturgeons. In such a situation, specific ascription that depends solely on head morphometry requires a clear understanding of the allometric trajectories and degree of interindiviudal variation for each species. However, I have shown elsewhere (Rincón, 2000) that, even in the near absence of true *A. naccarii* of adequate size for comparison, the standard techniques for treatment of allometry correctly identify size-caused gradients and do not separate large and small *A. sturio* as morphometrically distinct. On the other hand, the choice of a less-than-apt methodology for size-adjustment while disregarding other potentially diagnostic anatomical

traits (Holčík, 1989; Elvira and Almodóvar, 2000; can, of course, lead to confusion, as in Garrido-Ramos *et al.,* 1997).

The latter authors claimed that the value C - A discriminated between *A. sturio* (C - A ≤ 0) and *A. naccarii* (C - A > 0), and that this trait was size-independent (Garrido-Ramos *et al.,* 1997). However, simple algebra shows that the substraction of two size-dependent variables cannot be at the same time discriminating and size-independent (Rincón, 2000) and, unsurprisingly, C - A is in fact significantly correlated with Tl in both farmed *A. naccarii* and wild Iberian sturgeons (figure 2; $p < 0.001$). Moreover, C - A can be negative in small *A. naccarii* (table I, figure 2) and very similar in *A. sturio* and farmed *A. naccarii* of similar size (figure 2), being clearly inferior than measurements such as A, B or F .

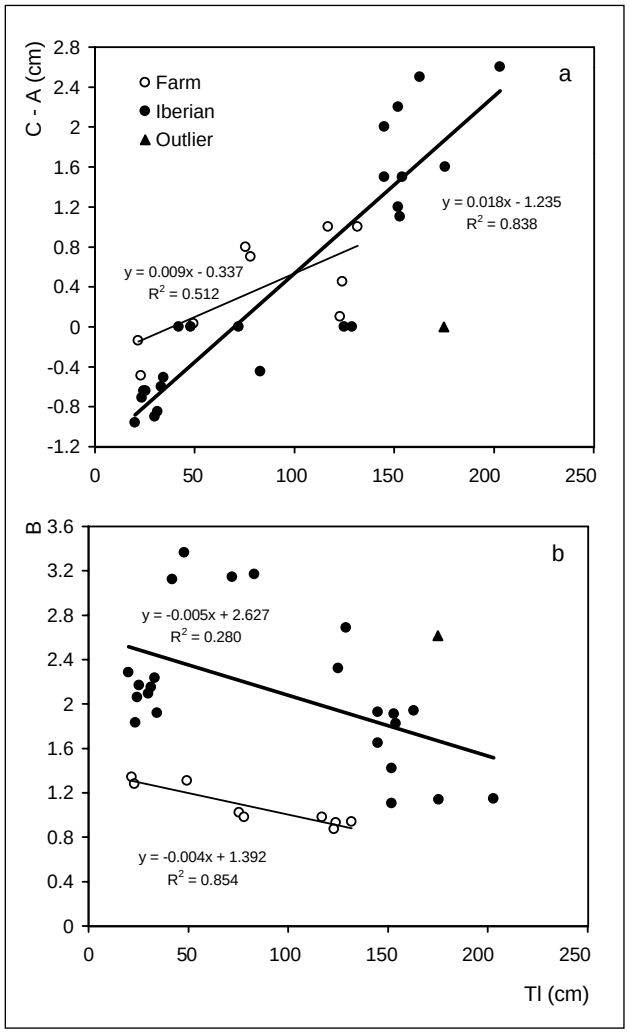

Figure 2. Scatterplots and regressions of (a) C - A (cm), and (b) F:B (dimensionless) against Tl (cm). Symbols as in figure 1

F:B, the other parameter Garrido-Ramos *et al.* used to separate *A. sturio* from *A. naccarii*, is, despite their claim to the contrary, also influenced by specimen size (figure 2; $p < 0.01$). As such, it offers no advantage over measurements such as A, B or F (figure 1), while being plagued by the problems derived from its being a ratio (Atchley, Gaskins and Anderson, 1976; Atchley and Anderson, 1978; Philips, 1983; Packard and Boardman, 1988; Prairie and Bird, 1989; Jackson and Somers, 1989) and not representing a distance between two identifiable features of sturgeon anatomy (Rincón, 2000).

Distribution in multivariate morphospace

The farmed *A. naccarii* specimens were clearly segregated from wild sturgeons taken in Iberian waters on the bivariate plane defined by the first two PCs extracted from the covariance matrix of *log*-transformed morphometric variables (figure 3).

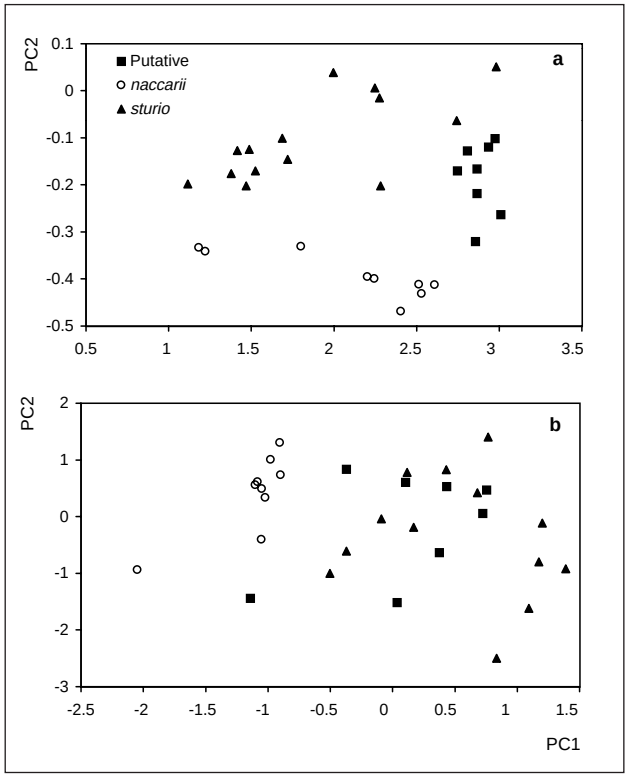

Figure 3. Sturgeon ordination on multivariate morphometric axes from PCAs using (a) the covariance matrix of *log*-transformed measurements, and (b) the correlation matrix of size-adjusted cephalic morphometric traits. Symbols denote specific ascription in Garrido-Ramos *et al.* (1997) and fish origin. Solid triangles: wild-caught *A. sturio*; solid squares, putative wild-caught *A. naccarii*; open circles: *A. naccarii* from the fish farm

This separation occurred along PC2 (5.41 % of variance; eigenvalue: 0.13) which reflected a gradient from longer, more slender snouts (positive end of the axis) towards those shorter and wider (negative end, figure 3a): B had the highest negative loading on it while those of A, C and F were all positive (table III). In contrast, putative wild *A. naccarii*, as recognised by Garrido-Ramos *et al.* (1997),

Table III. Eigenvalues and proportions of variance explained of the first three principal components (PC1-PC3) extracted from the covariance matrix and loadings of the original variables on them

	PC1	PC2	PC3
log Tl	0.97	–0.13	–0.20
log A	0.92	0.37	0.03
log B	0.92	–0.35	0.26
log C	0.98	0.16	< 0.001
log F	0.96	0.27	0.03
log LFR	0.99	–0.04	–0.02
Eigenvalue	2.28	0.13	0.05
% Variance	91.9	5.41	1.87

and the other wild sturgeons overlapped extensively along PC2, and only displayed some separation along PC1 (figure 3a). As often happens (Humphries *et al.,* 1981; Bookstein *et al.,* 1985; Rohlf and Bookstein, 1987; Klingenberg, 1996), PC1 was both a general size factor (the loadings and coefficients of the original variables on it were all positive and of a similar magnitude; table III) and the major gradient in the morphospace defined by the current sample (92 % of total variance explained; eigenvalue: 2.28). Component 3 explained little variance (1.87 %) and had a small eigenvalue (0.05), and is not discussed further. Subsequent components were even less relevant.

Therefore, PC2 corresponds to the morphometric traits of the head acknowledged to differentiate *A. sturio* and *A. naccarii* (Holčík, 1989; Garrido-Ramos *et al.,* 1997), and it clearly separated those confirmed *A. naccarii* from the homogenous group formed by all wild Iberian fish, without any gap between the *A. sturio* and the supposed *A. naccarii* of Garrido-Ramos *et al.* (1997), whose only detected differentiation was in size (PC1). These findings replicate almost exactly, being, if anything, clearer than, my previous ones for a sample including only one confirmed *A. naccarii* (Rincón, 2000), and thus further validate them and the conclusions I derived therefrom. Besides, they are also completely consistent with the results of the exploration of head allometry presented immediately above. It is noteworthy that group assignment in that analysis was not based on morphometric traits (e.g. farm vs wild fish) and that the PCA entailed no *a priori* group ascription.

Wild Iberian sturgeons and farmed *A. naccarii* were again segregated on the plane defined by PC1 and PC2 obtained from the correlation matrix of the size-adjusted variables. In contrast, putative wild *A. naccarii* and *A. sturio* overlapped extensively, and showed no spatial separation (figure 3b). Most of the segregation was now along PC1 (figure 3b), which explained 70 % of the variance and had an eigenvalue of 3.49. PC1 reflected the same narrower vs wider gradient in head morphology as PC2 from the covariance analysis, and shared with the latter a substantially similar structure as indicated by the variable loadings on them (tables III and IV). PC2 arranged fish within each group (farmed and wild fish) along it according to their snout width (high loading of B), but explained less variance (16 %) than any of the original variables *per se*, as indicated by its eigenvalue < 1 (table IV). Further components had even lower eigenvalues, and proportions of explained variance and are not presented.

Table IV. Eigenvalues and proportions of variance explained of the first two principal components (PC1-PC2) extracted from the correlation matrix of size-adjusted variables matrix and loadings of the original variables on them

	PC1	PC2
log A_{adj}	0.96	0.04
log B_{adj}	–0.54	0.82
log C_{adj}	0.90	0.16
log F_{adj}	0.96	0.04
log LFR_{adj}	0.73	0.30
Eigenvalue	3.49	0.80
% Variance	69.8	15.9

Therefore, the morphological gradient that PC2 from the covariance matrix identified appears regardless of the method of PC extraction and with both *log*-transformed or size-adjusted variables. It also becomes the major one, capturing most of the remaining variance once the effect of size is re-

moved, and, again, such a gradient neatly separates farmed *A. naccarii* from wild Iberian sturgeons, while it does not distinguish groups within the sample of wild fish. This confirms the results of the previous analyses in present paper. So far, univariate and multivariate examination of the patterns of allometry among morphometric measurements and multivariate ordination of specimens in relatively size-free morphospace has provided no evidence of the presence of two morphologically dissimilar groups among sturgeons caught in Iberian waters during the last century. On the other hand, the aforesaid methods have clearly identified known *A. naccarii* coming from a fish farm as distinct from the remaining fish captured in the wild in waters of the Iberian Peninsula.

These results agree completely with my findings (Rincón, 2000), but run counter to those of Garrido-Ramos *et al.* (1997). I have attributed this discrepancy to the fact that Garrido-Ramos *et al.* (1997) based their results on C - A and F:B without any correction of those parameters for size effects (Rincón, 2000), and the findings presented here provide additional support for this contention, as well as by presenting further instances of C - A and F:B having statistically significant correlation with size, and showing how supposedly diagnostic, species-specific values of C - A are, in fact, size-dependent.

A MANOVA performed on the five original, size-adjusted variables found significant differences between groups (Pillai's trace: 1.023; Wilk's $\lambda = 0.155$; df = 10, 50; $p < 0.0001$) for *log* A_{adj}, *log* B_{adj}, *log* C_{adj}, *log* F_{adj}, ($F_{2,29} = 3.71$-14.83; $p = 0.03$-0.00004 for each individual variable), but not *log* LFR_{adj} ($F_{2,29} = 0.40$; $p = 0.68$). Hence, only the first four variables were then submitted to the DFA. The DFA elicited statistically significant between-group discrimination ($F_{8,52} = 7.17$; $p < 0.0001$) and extracted two canonical variates (CV). Only the first, CV1, was statistically significant and had a high eigenvalue (table V). Along it, farmed *A. naccarii* were clearly separated from wild Iberian sturgeons, whereas wild *A. sturio* and putative wild *A. naccarii* overlapped extensively (figure 4). CV2, in contrast, was not significant: its eigenvalue was very low, and provided no group separation (figure 4, table V).

This distinction between farm sturgeons and both groups of wild fish was highly statistically significant according to the F statistics associated with the squared Mahalanobis distances (SMD) between the respective group centroids on the plane CV1-CV2 (farmed-putative *A. naccarii*: SMD = 10.07, $F_{4,26} = 9.02$; $p = 0.00011$; farmed *A. sturio*: SMD = 17.42; $F_{4,26} = 19.34$; $p < 0.00001$). On the other hand, the two putative groups of wild fish were not significantly separated (putative *A. naccarii*-*A. sturio*: SMD = 1.63; $F_{4,26} = 1.81$; $p = 0.16$). Reflecting this lack of differences, the DFA classified 44 % (4 specimens) of the putative *A. naccarii* as *A. sturio* and 21 % (3 fish) of the *A. sturio* as putative *A. naccarii*. However, it correctly assigned the remaining 11 *A. sturio* (79 %) and all farmed fish to their respective *a priori* groups. Only one claimed wild *A. naccarii* was mistakenly grouped with the farmed sturgeon. This

Table V. Eigenvalues, statistical significance (Wilk's λ associated probability) and variable loadings for the two canonical variates (CV) extracted by a DFA of size-adjusted morphometric variables that exhibited significant between group differences in a previous MANOVA

	CV1	CV2
log A_{adj}	0.80	0.02
log B_{adj}	–0.38	0.61
log C_{adj}	0.43	0.26
log F_{adj}	0.82	0.34
Eigenvalue	3.04	0.09
Wilk's λ	69.8	15.9
p	< 0.00001	0.484

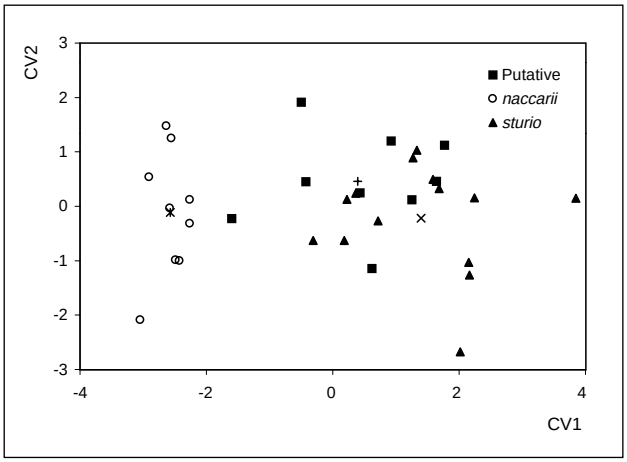

Figure 4. Sturgeon ordination on the plane defined by the two canonical variates (CV1, CV2) extracted by a DFA of size-adjusted, morphometric measurements of the snout. Symbols denote specific ascription in Garrido-Ramos *et al.* (1997) and fish origin. (*), (+) and (×) mark group centroid position for *A. naccarii* from the fish farm, putative wild-caught *A. naccarii* and wild-caught *A. sturio*, respectively. Remaining symbols as in figure 2

was the largest fish in the sample (Tl = 203 cm), specimen MUC1 in Garrido-Ramos *et al.* (1997), a stuffed specimen captured in the Tagus River (i.e., not from the Guadalquivir basin) in 1890, and which other authors have identified as *A. sturio* according to all other anatomical traits (Almaça, 1988; Elvira, pers. comm.).

CV1 reflected, once more, the difference between the narrower snouts of the wild Iberian sturgeons and the wider ones of the farmed *A. naccarii*. All size-adjusted longitudinal measurements of the snout had positive loadings on it (particularly *log* A_{adj} and *log* F_{adj}), while the snout width's (*log* B_{adj}) loading was negative (table V). Therefore, the results of the DFA mirror and reinforce those of all previous analyses. Even DFA, a technique designed to maximise between-group discrimination, fails to uncover any significant dissimilarity in head morphology between the two supposed groups of sturgeons from Iberian rivers, once the effect of size is removed. At the same time, DFA clearly detects a highly significant difference in snout shape between all wild fish and the captive *A. naccarii*.

Concluding remarks

Extensive multivariate and univariate exploration that adequately accounts for ontogenetic allometry's effects on the morphometric data in Garrido-Ramos *et al.* (1997) fails to provide any evidence suggestive of the presence of two morphologically dissimilar groups in those sturgeons captured in rivers of the Iberian Peninsula throughout the last century and housed in different scientific collections. These same techniques, however, do adequately identify and separate the farmed *A. naccarii* specimens (acquired at a fish farm that raises sturgeons from Italian stock) from their wild-caught Iberian counterparts. Size is the major axis of morphometric variation within the sample, but, once its effect is statistically removed, a gradient in head shape that provides no discrimination between the two putative groups of wild-caught sturgeons, but that neatly separates farmed fishes from the rest, becomes readily apparent. I had previously identified that gradient (Rincón, 2000), but in my previous paper, due to the insufficient representation of *A. naccarii* in the database (both in terms of number and size range), it represented a smaller proportion of the total morphometric variance, and was weaker and somewhat less clear than in the present paper.

Therefore, the findings and conclusions presented above confirm and support those of my previous work (Rincón, 2000). Both, however, directly contradict those of Garrido-Ramos *et al.* (1997). This discrepancy is largely resolved by the examination of the methodology that produced the evidence that allegedly supported the claims of morphometric evidence proving the past presence of wild *A. naccarii* in the Guadalquivir. As has been shown both here (e.g. figure 2) and elsewhere (Rincón, 2000), Garrido-Ramos *et al.* (1997) employed procedures unable to deal with ontogenetic allometry and, as a consequence, they incorrectly identified larger specimens of *A. sturio* as *A. naccarii*. Again, I must note that according to these authors there would be no *A. naccarii* smaller than 145.00 cm in a sample where putative conspecifics comprise 75% of all adults; an unusual pattern in the light of what is known of the biology and migratory behavior of sturgeons (Holčík, 1989). At face value, this would mean that *A. sturio* is the only species that ever reproduced in Iberian rivers, while *A. naccarii* never did, despite their adults having been apparently quite common.

However, according to my results, the real situation is rather less intriguing: the results of Garrido-Ramos *et al.* (1997) are a methodological artifact, and there is no morphometric evidence to consider *A. naccarii* native to the Iberian Peninsula. Therefore, decisions concerning the management of Adriatic sturgeon stocks in Spain should always bear present Birstein *et al.* (1998) recommendations for farmed sturgeon species in Western Europe: "[farmed sturgeons]... should not be allowed to escape into the wild and should never ever be released."

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